

**We claim:**

1. A self-referencing colorimetric resonant optical biosensor comprising:
  - 5 (a) a liquid holding vessel comprising a colorimetric resonant optical biosensor as a surface;
  - (b) a fluid-impermeable divider on the colorimetric resonant optical biosensor surface, wherein the fluid impermeable divider separates the liquid holding vessel into two or more assay regions, wherein the fluid-impermeable divider segregates  
10 an immobilization volume within each assay region, and wherein the fluid-impermeable divider allows a reaction volume to encompass all assay regions in the liquid-holding vessel.
2. The self-referencing colorimetric resonant optical biosensor of claim 1, wherein one or more specific binding substances are immobilized on one or more of the assay  
15 regions to form one or more reaction regions, and wherein no specific binding substances are immobilized on one or more of the assay regions to form one or more reference regions.
3. The self-referencing colorimetric resonant optical biosensor of claim 2, wherein when the biosensor is illuminated a resonant grating effect is produced on the reflected  
20 radiation spectrum and wherein the depth and period of a grating of the biosensor are less than a wavelength of the resonant grating effect.
4. The self-referencing colorimetric resonant optical biosensor of claim 2, wherein a narrow band of optical wavelengths is reflected from the biosensor when the biosensor is illuminated with a broad band of optical wavelengths.

5. The self-referencing colorimetric resonant optical biosensor of claim 2, wherein the one or more specific binding substances are bound to their specific binding partners.
6. The self-referencing colorimetric resonant optical biosensor of claim 1, wherein the liquid-holding vessel is selected from the group consisting of a microtiter plate well, a test tube, a Petri dish and a microfluidic channel.
7. The self-referencing colorimetric resonant optical biosensor of claim 1, wherein the biosensor comprises two or more liquid-holding vessels.
8. The self-referencing colorimetric resonant optical biosensor of claim 7, wherein one or more specific binding substances are immobilized on one or more of the assay regions to form one or more reaction regions, and wherein no specific binding substances are immobilized on one or more of the assay regions to form one or more reference regions.
9. A self-referencing colorimetric resonant optical biosensor comprising:
- (a) one or more liquid-holding vessels comprising a colorimetric resonant optical biosensor as a surface; and
  - (b) one or more specific binding substances immobilized on a first portion of the colorimetric resonant optical biosensor of each liquid-holding vessel forming a reaction surface, and no specific binding substances immobilized on a second portion of the colorimetric resonant optical biosensor of each liquid-holding vessel forming a reference surface.
10. The self-referencing colorimetric resonant optical biosensor of claim 9, wherein the biosensor comprises two or more reaction surfaces in each liquid holding vessel and two or more reference surfaces in each liquid holding vessel.

11. The self-referencing colorimetric resonant optical biosensor of claim 9, wherein when the biosensor is illuminated a resonant grating effect is produced on the reflected radiation spectrum and wherein the depth and period of a grating of the biosensor are less than a wavelength of the resonant grating effect.
- 5 12. The self-referencing colorimetric resonant optical biosensor of claim 9, wherein a narrow band of optical wavelengths is reflected from the biosensor when the biosensor is illuminated with a broad band of optical wavelengths.
13. The self-referencing colorimetric resonant optical biosensor of claim 9, wherein the one or more specific binding substances are bound to their specific binding partners.
- 10 14. The self-referencing colorimetric resonant optical biosensor of claim 9, wherein the liquid-holding vessel is selected from the group consisting of a microtiter plate well, a test tube, a Petri dish and a microfluidic channel.
15. A method of making a self-referencing colorimetric resonant optical biosensor comprising:
- 15 (a) immobilizing one or more specific binding substances in an immobilization volume to one or more assay regions of a self-referencing colorimetric resonant optical biosensor, wherein the biosensor comprises a liquid holding vessel comprising a colorimetric resonant optical biosensor as a surface, and a fluid-impermeable divider on the colorimetric resonant optical biosensor surface, wherein the fluid impermeable divider separates the liquid holding vessel
- 20 into two or more assay regions, wherein the fluid-impermeable divider segregates an immobilization volume within each assay region, and wherein the fluid-

impermeable divider allows a reaction volume to encompass all assay regions in the liquid-holding vessel; and

(b) preserving one or more assay regions as one or more reference regions, wherein the one or more reference regions do not contain immobilized specific binding substances.

16. A method of making a self-referencing colorimetric resonant optical biosensor comprising immobilizing one or more specific binding substances on a first portion of a surface of a colorimetric resonant optical biosensor forming a reaction region, wherein a second portion of the colorimetric resonant optical biosensor contains no specific binding substances forming a reference region, wherein the colorimetric resonant optical biosensor comprises a surface of a liquid-holding vessel.

17. A method of detecting the binding of one or more specific binding substances to their respective binding partners in the self-referencing colorimetric resonant optical

biosensor of claim 2 comprising:

(a) applying one or more specific binding partners in a reaction volume to the liquid holding vessel of claim 2;

(b) illuminating the one or more reaction regions and the one or more reference regions with light;

(c) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the one or more reaction regions and the one or more reference regions; and

(d) comparing the maxima or minima of the one or more reference regions to the maxima or minima from the one or more reaction regions;  
wherein the binding of one or more specific binding substances to their respective binding partners is detected.

5 18. A method of detecting the binding of one or more specific binding substances to their respective binding partners in a self-referencing colorimetric resonant optical biosensor of claim 9 comprising:

(a) applying one or more specific binding partners in a reaction volume to the one or more liquid holding vessels of claim 9;

10 (b) illuminating the reaction surfaces and the reference surfaces with light;

(c) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the reaction surfaces and the reference surfaces;  
and

(d) comparing the maxima or minima of the one or more reference surfaces to  
15 the maxima or minima from the one or more reaction surfaces;

wherein the binding of one or more specific binding substances to their respective binding partners is detected.

19. A method of detecting activity of an enzyme in a self-referencing colorimetric resonant optical biosensor of claim 2 comprising:

20 (a) applying one or more enzymes in a reaction volume to the liquid holding vessel of claim 2;

(b) illuminating the one or more reaction regions and the one or more reference regions with light;

- (c) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the one or more reaction regions and the one or more reference regions; and
- (d) comparing the maxima or minima of the one or more reference regions to the
- 5           maxima or minima from the one or more reaction regions;
- wherein the activity of an enzyme is detected.

20.    A method of detecting activity of an enzyme in a self-referencing colorimetric resonant optical biosensor of claim 9 comprising:

- (a)    applying one or more enzymes in a reaction volume to the one or more
- 10           liquid holding vessels of claim 9;
- (b)    illuminating the one or more reaction surfaces and the one or more reference surfaces with light;
- (c)    detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the one or more reaction surfaces and the one or
- 15           more reference surfaces; and
- (d)    comparing the maxima or minima of the one or more reference surfaces to the maxima or minima from the one or more reaction surfaces;
- wherein the activity of an enzyme is detected.

21.    A method of detecting the inhibition activity of one or more molecules against

20   one or more enzymes or specific binding partners in a self-referencing colorimetric resonant optical biosensor of claim 2 comprising:

- (a)    applying one or more molecules suspected of having inhibition activity in a reaction volume to the liquid holding vessel of claim 2;

- (b) applying one or more enzymes or specific binding partners in a reaction volume to the liquid holding vessel;
- (c) illuminating the one or more reaction regions and the one or more reference regions with light;
- 5 (d) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the one or more reaction regions and the one or more reference regions; and
- (e) comparing the maxima or minima of the one or more reference regions to the maxima or minima from the one or more reaction regions;
- 10 wherein the inhibition activity of one or more molecules is detected.
22. A method of detecting inhibition activity of one or more molecules against one or more enzymes or specific binding partners in a self-referencing colorimetric resonant optical biosensor of claim 9 comprising:
- (a) applying one or more molecules suspected of having inhibition activity in
- 15 a reaction volume to the one or more liquid holding vessels of claim 9;
- (b) applying one or more enzymes or specific binding partners in a reaction volume to the one or more liquid holding vessels;
- (c) illuminating the one or more reaction surfaces and the one or more reference surfaces with light;
- 20 (d) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the one or more reaction surfaces and the one or more reference surfaces; and

- (e) comparing the maxima or minima of the one or more reference surfaces to the maxima or minima from the one or more reaction surfaces;  
wherein the inhibition activity of one or more molecules is detected.